

Aedes aegypti (L.) and *Aedes albopictus* (Skuse) in Singapore City

5. Observations in Relation to Dengue Haemorrhagic Fever

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Dengue haemorrhagic fever in Singapore was a disease of the urban human population, with concentrations of cases occurring in areas of high population density. Mosquito surveys revealed that these areas also had high population densities of Ae. aegypti and Ae. albopictus.

The disease occurred throughout the year but the incidence of cases appeared to follow a seasonal pattern. Observations from 1966 to 1968 showed that the number of cases increased in April, reached a peak in November, and, thereafter, decreased until the next increase in April of the following year. The epidemic curve generally agreed with the fluctuations of both Ae. aegypti and Ae. albopictus populations, although the latter species appeared to show a better correspondence with the incidence of cases.

Six dengue viruses were isolated from the two Aedes species during 1966. One dengue type 2 virus was isolated from a pool of Ae. aegypti and 1 dengue type 1 virus and 4 dengue type 2 viruses were recovered from 5 pools of Ae. albopictus. These viruses were isolated from mosquitos collected during the period of increase in the incidence of cases and in 4 different areas of the city. The dengue virus infection rates per 1 000 mosquitos estimated in the present study were 0.51 for Ae. aegypti and 0.59 for Ae. albopictus.

The data obtained in the present study suggest that both Ae. aegypti and Ae. albopictus are involved in the transmission of dengue haemorrhagic fever in Singapore.

Since the first outbreak in 1960, epidemics of dengue haemorrhagic fever have recurred annually in Singapore (Chan, Lim & Ho, 1967). The results of a mosquito survey carried out at the end of the 1960 outbreak (from November 1960 to January 1961) suggested that *Aedes aegypti* was probably the vector of the disease (Lim, Rudnick & Chan, 1961). This species was common in the urban area, in as many as 50% of the houses visited, and its distribution was consistent with that of the dengue

haemorrhagic fever cases, which had an urban and suburban distribution. This conclusion was subsequently supported by the recovery of dengue viruses from mosquitos. Of the 6 strains of dengue type 2 virus isolated, 5 were from pools of *Ae. aegypti* and one from a pool of *Aedes albopictus* (Rudnick & Chan, 1965).

During the 1960-61 mosquito survey it was also noted that *Ae. albopictus* was common in both urban and rural areas. With the subsequent isolation of dengue virus from this species it was felt that its vector position in relation to dengue haemorrhagic fever needed further study. *Ae. albopictus* has been shown to be an efficient vector in the experimental transmission of classical dengue (Simmons, St. John & Reynolds, 1931) and has been considered as an important vector of endemic dengue in South-East Asia (Smith, 1956). Mosquito surveys in the city were therefore initiated in 1966 to study the bionomics of *Ae. aegypti* and *Ae. albopictus* and their

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vector status in dengue haemorrhagic fever. The results of part of the study have been presented elsewhere (Chan, Y. C. et al., 1971; Chan, Ho & Chan, 1971; Ho, Chan & Chan, 1971; Chan, K. L. et al., 1971). This paper describes the distribution, population density, and fluctuations of the two *Aedes* species in relation to the incidence of dengue haemorrhagic fever and the results of virus isolations from the mosquitoes. A preliminary report of some of the data has already been published (Chan, Lim & Ho, 1967).

METHODS

Incidence of dengue haemorrhagic fever

It is difficult to determine the incidence of dengue haemorrhagic fever as the disease is not notifiable in Singapore. Since the first outbreak of the disease in 1960, however, the Department of Bacteriology of the University of Singapore has regularly received blood specimens from hospital patients with a clinical diagnosis of dengue haemorrhagic fever. The specimens were voluntarily submitted by clinicians for laboratory confirmation of the disease and thus did not represent the total number of dengue haemorrhagic fever patients in the hospitals.

The sera from patients were routinely examined by the complement-fixation test for evidence of a recent infection with dengue virus or group-B arbovirus. Virus isolation was also attempted in a proportion of acute-phase sera. Many of the sera received were single specimens (acute phase) and some of the paired sera (acute and convalescent) were not obtained at an adequately spaced interval for laboratory confirmation to be established. However, the number of these sera that were dengue positive was estimated by extrapolation from the percentage positive for dengue of those paired sera that were suitable for serological testing. In the estimation, it was assumed that the proportion of sera positive for dengue antibodies in the group not tested was the same as that found in the sera that were tested.

In this paper the total number of laboratory confirmed dengue haemorrhagic fever patients seen at 4 general hospitals in the city is presented to represent the incidence of the disease. This is a minimum number since not all hospitalized patients have been tested. It appears to project the true epidemic pattern of the disease as the pattern was consistent for 3 consecutive years, 1966, 1967, and 1968 (Fig. 2-4).

Virus isolation from mosquitoes

Adult female *Ae. aegypti* and *Ae. albopictus* collected from the field were identified and grouped

according to species, collecting stations, and date of collection. They were stored at -60°C in ampoules plugged with cotton wool until inoculated.

Virus isolation was carried out in 1-day-old mice. For inoculation, the mosquitoes were divided into pools, 25-45 mosquitoes per pool, according to the date and place of collection, whenever possible. They were suspended in phosphate-buffered saline, pH 7.4-7.6, containing 200 IU of penicillin, 200 mg of streptomycin, and 0.75% bovine plasma albumin. The mosquitoes were ground in 0.04 ml of diluent per mosquito, and the suspension was centrifuged in the cold at 2500 rev/min for 25 min. A 0.3-ml volume of the supernatant was inoculated into each mouse by a combined subcutaneous and intracerebral route. Each pool of mosquitoes was inoculated into 6-8 mice, which were observed for 21 days in a mosquito-proof room. Inoculated mice that did not show any obvious signs of sickness were "blind" passed 2-3 times at 10-day intervals. Reisolation of virus was attempted in all cases from the original mosquito suspension.

All processing and inoculation work was carried out in a room where no known arbovirus or other virus had been handled. Inoculated mice for initial virus isolation were not kept in the same room as mice inoculated with known viruses or viruses under adaptation.

Virus isolates were identified by cross-complement-fixation test, and in some cases also by the agar-gel microprecipitin technique (Chan, 1965).

*Distribution, population density, and fluctuations of *Ae. aegypti* and *Ae. albopictus**

The data included in this paper have already been presented elsewhere (Chan, Y. C. et al., 1971; Chan, Ho & Chan, 1971; Ho, Chan & Chan, 1971).

RESULTS

*Distribution of dengue haemorrhagic fever cases and *Aedes* populations*

Fig. 1 shows the distribution of dengue haemorrhagic fever cases mapped by residence between 1960 and 1966, and some of the areas where mosquito surveys have been carried out. The cases from 1960 to 1964 were taken from one hospital, while those in 1966 were from 4 hospitals. It can be seen that dengue haemorrhagic fever was a disease of the urban human population, with concentrations of cases in certain areas having a high density of human population. Five such areas were apparent

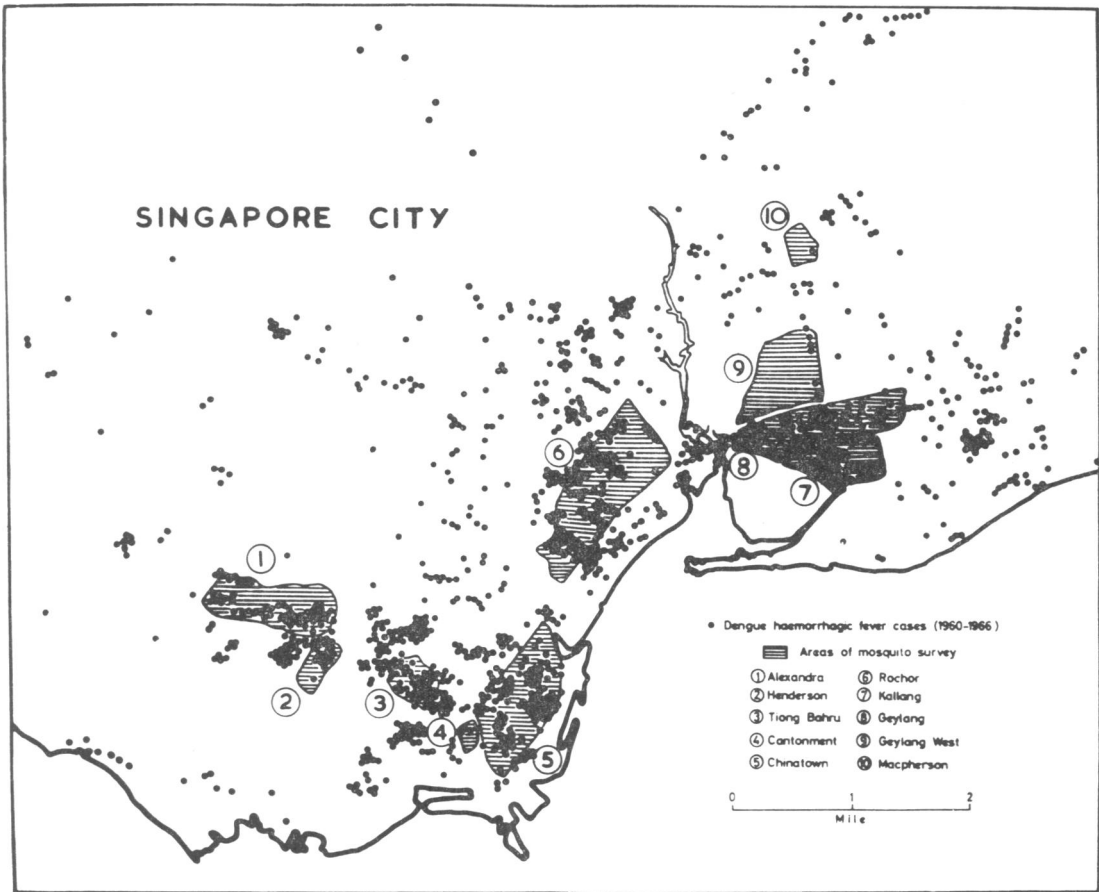


Fig. 1. Singapore city, showing the distribution of dengue haemorrhagic fever cases and the larval survey areas.
Conversion factor for scale: 1 mile = 1.6 km.

and these also coincided with areas where both *Ae. aegypti* and *Ae. albopictus* have been found to be widely distributed and abundant. These areas were Geylang, Rochor, Chinatown, Tiong Bahru, and Henderson/Alexandra (Chan, Y. C. et al., 1971). The predominant species was *Ae. aegypti* and the premise indices in four of the areas ranged from 14.8 to 29.6. These premise indices were probably the highest to be found in the city. The population density of *Ae. aegypti* was also high in these areas, as shown by the average number of larvae per housing unit estimated for each area. It ranged from 1 larva per house in Tiong Bahru to 8 larvae per house in Henderson.

Aedes albopictus, though less widely distributed than *Ae. aegypti*, was found in all the city areas

surveyed. The mean premise index estimated for this species in 10 different areas was 2.5, with Henderson having the highest index of 15.9. The population density of this species in those areas where the dengue haemorrhagic fever cases were concentrated ranged from 4 larvae per house to 1 larva in 4 houses. This population was equally distributed indoors and out of doors, as shown by the study of breeding habitats (Chan, Ho & Chan, 1971).

In some areas, the house-associated population of *Ae. albopictus* represented only a fraction of the entire population in the survey area. In these areas, this species was found in foci of high density in open spaces such as vacant land, open fields, and parks. Such open spaces were not uncommon within the city area and breeding of this species was

often found in dumps of rubber tires and tin cans. In Rochor, for instance, the open-space population accounted for nearly 80% of the total *Ae. albopictus* population in the area.

Incidence of dengue haemorrhagic fever and Aedes population fluctuations

Dengue haemorrhagic fever cases occurred throughout the year but the incidence of cases appeared to follow a seasonal pattern (Figs. 2-4). The incidence of cases in the 3 years 1966, 1967, and 1968 showed an increase in April and reached a peak in November after a slight drop from July to October. The incidence dropped again in December and the low level continued until the next increase in April of the following year.

Fig. 2 shows the epidemic curve of 1966 and the fluctuations of population density of *Ae. aegypti* and *Ae. albopictus* observed in the same year. There was a general correspondence between the epidemic curve and the population fluctuations of the two species. The simultaneous rise in the populations of the two mosquitos in February-March was followed by an increase in the incidence of cases, which continued to rise sharply in the subsequent months. The sharp increase in the number of cases coincided with a second rise in the populations of *Ae. aegypti* and *Ae. albopictus* from May to July. The *Ae. albopictus* population rose earlier and attained a peak earlier

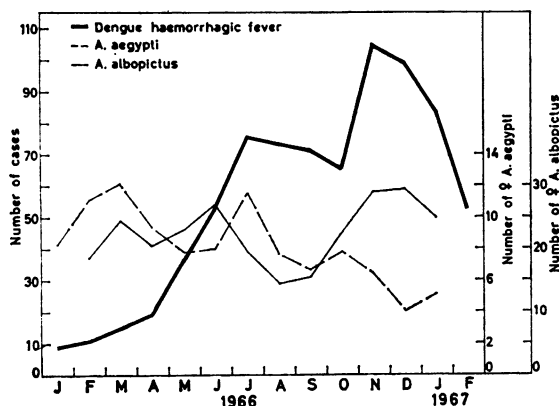


Fig. 2. The 1966 incidence of dengue haemorrhagic fever and the population fluctuations of *Ae. aegypti* and *Ae. albopictus*. Incidence was estimated on the basis of the number of hospitalized patients who had a recent dengue virus or group B arbovirus infection confirmed serologically. The population fluctuations of each of the two mosquito species are the mean of the fluctuations observed in 3 different city areas (see Ho, Chan & Chan, 1971).

than the *Ae. aegypti* population. The slight fall in the incidence of cases from July to October was associated with the fall in the populations of the two species. The incidence increased again in November with the rise in the mosquito populations and the numbers of both cases and mosquitos fell again in December.

The epidemic curve appeared to correspond better with the population fluctuations of *Ae. albopictus* than with those of *Ae. aegypti*. This was especially apparent during the peak incidence of cases in November, when the rise in the *Ae. aegypti* population was comparatively small; the rise in *Ae. albopictus* population at this time was proportional to the incidence of cases.

Studies of the relationship between the incidence of cases and the population fluctuations of *Ae. albopictus* between 1966 and 1967 (Fig. 3) and of *Ae.*

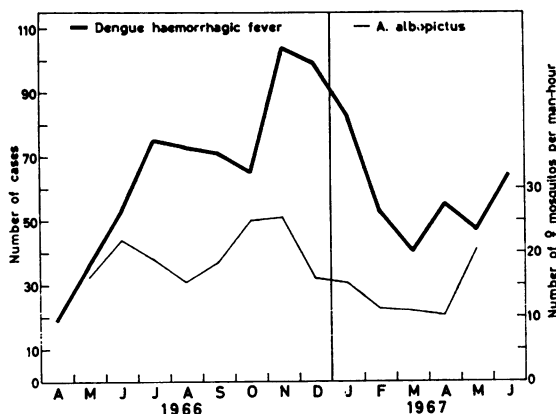


Fig. 3. The 1966-67 incidence of dengue haemorrhagic fever and the population fluctuations of *Ae. albopictus* in McAlister Road. Incidence was estimated on the basis of the number of hospitalized patients who had a recent dengue virus or group B arbovirus infection confirmed serologically. The location of the *Ae. albopictus* study station has been noted by Ho, Chan & Chan (1971)

aegypti from 1967 to 1968 (Fig. 4) generally agreed with the observations of 1966. The 1966-67 study of *Ae. albopictus* populations was carried out in an entirely different area but the fluctuations observed were similar to those seen in 1966. The incidence of cases was closely associated with the fluctuations of this species. The population fluctuations of *Ae. aegypti*, on the other hand, were less well correlated with the incidence of cases.

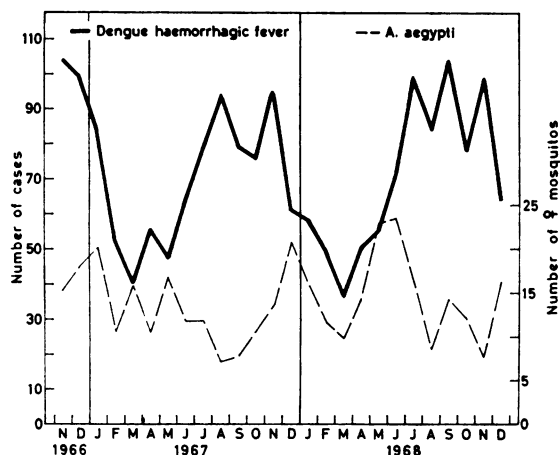


Fig. 4. The 1967-68 incidence of dengue haemorrhagic fever and the population fluctuations of *Ae. aegypti* in Geylang. The incidence was estimated on the basis of the number of hospitalized patients who had a recent dengue virus or group B arbovirus infection serologically. The location of the *Ae. aegypti* study station has been noted by Ho, Chan & Chan (1971).

Dengue viruses isolated from *Ae. aegypti* and *Ae. albopictus*

Female mosquitos of the two *Aedes* species collected in 1966 from 7 areas in the city (Ho, Chan & Chan, 1971) were inoculated into suckling mice for the isolation of virus. A total of 1961 *Ae. aegypti* and 8475 *Ae. albopictus* was divided into pools and inoculated. One dengue type 2 virus was isolated from a pool of *Ae. aegypti*, and from 5 pools of *Ae. albopictus* 1 dengue type 1 virus and 4 dengue type 2 viruses were recovered (Table 1). The single virus isolated from *Ae. aegypti* was not reisolated

but 2 of the dengue type 2 isolates were successfully reisolated. All 6 dengue viruses were isolated from mosquitos collected between April and October, during the period of increase in the incidence of dengue haemorrhagic fever. The pools of *Ae. albopictus* that yielded dengue viruses came from all the 4 collecting stations located in different parts of the city. Based upon the present data, the dengue virus infection rates per 1000 mosquitos were 0.51 for *Ae. aegypti* and 0.59 for *Ae. albopictus*.

DISCUSSION

It has been established that *Ae. aegypti* is the primary vector of dengue haemorrhagic fever in areas where the disease has been reported (*Bull. Wild Hlth Org.*, 1966). The conclusion seems to have been based principally on two findings: (1) the consistent distribution of dengue haemorrhagic fever cases and of *Ae. aegypti*, and (2) the isolation of dengue viruses from naturally infected *A. aegypti* mosquitos. A further reason for the conclusion seems to be the assumption that, since the disease has a dengue virus etiology, a similar vector species, if it were present, would be involved. Thus, Rudnick et al. (1965), in reporting the disease in Malaysia (Penang) stated, "Mosquito surveys on Penang Island revealed the presence of *A. aegypti* mosquitoes in relatively high abundance in the urban areas. *A. albopictus* mosquitoes were present in high abundance throughout the island in urban, rural, and forest areas. Although no virus isolations were made from the collected mosquitoes, *A. aegypti* was considered the primary vector of the disease on epidemiological grounds. The distribution of the known cases coincided with the distribution of *A. aegypti* but not with that of any other mosquito

Table 1. Dengue viruses isolated from *Aedes* mosquitos, 1966

Mosquito species	Date collected	Area	Virus isolate	Identification
<i>Ae. aegypti</i>	June	Rayman Ave./Dickson Road	M-68	Dengue type 2
<i>Ae. albopictus</i>	April	Lorong 29/McAlister Road	M-16 ^a	Dengue type 2
<i>Ae. albopictus</i>	July	McAlister Road	M-111	Dengue type 1
<i>Ae. albopictus</i>	August	Lorong 29	M-176	Dengue type 2
<i>Ae. albopictus</i>	October	College Road	M-228 ^a	Dengue type 2
<i>Ae. albopictus</i>	October	McNair Road	M-241	Dengue type 2

^a These viruses were successfully reisolated from the original mosquito suspension.

species in Penang. *A. aegypti* is the vector incriminated in other areas where haemorrhagic fever caused by dengue viruses has occurred."

The status of *Ae. albopictus* as a vector of dengue haemorrhagic fever in areas where this species is also present in high numbers in the urban area is uncertain. This species has been found to be common in the urban or city area during investigations of the epidemics in Manila, Philippines (Rudnick & Hammon, 1960, 1961), in Singapore (Lim, Rudnick & Chan, 1961), and in Penang, Malaysia (Rudnick et al., 1965). One strain of dengue type 2 virus has been isolated from this species collected towards the end of the 1960 epidemic in Singapore (Rudnick & Chan, 1965). Rudnick (1966), however, concluded that *Ae. albopictus* was not a vector of dengue haemorrhagic fever in these areas because its distribution did not correspond with that of the cases. He suggested that this species might be a vector of mild endemic dengue, whose distribution might be different from that of dengue haemorrhagic fever.

The present study of the distribution, population density, and annual fluctuations of *Ae. aegypti* and *Ae. albopictus* in urban areas during epidemics of dengue haemorrhagic fever showed that in Singapore both species were involved in the transmission of dengue viruses. A preliminary account and the conclusions of this study have already been reported (Chan, Lim & Ho, 1967). Both mosquito species were common and present in high density in areas of high human population density where concentrations of dengue haemorrhagic fever cases also occurred. *Ae. aegypti* had a higher population

density than *Ae. albopictus* in most of the areas surveyed. Breeding of *Ae. albopictus* indoors was very common and in areas where there were open spaces foci of intense breeding were found. The population fluctuations of the two species studied over 3 years showed correlation with the incidence of disease. However, the incidence of cases appeared to correlate better with the fluctuations of *Ae. albopictus*.

During the 1966 epidemic, 6 dengue viruses were isolated from these two species, 1 dengue type 2 virus was isolated from *Ae. aegypti*, and 1 dengue type 1 and 4 dengue type 2 viruses were isolated from *Ae. albopictus*. All 5 dengue viruses from *Ae. albopictus* were isolated from mosquitos collected in different areas of the city. The dengue virus infection rates per 1000 mosquitos, based on the present data, were 0.51 for *Ae. aegypti* and 0.59 for *Ae. albopictus*. These infection rates were much lower than those obtained in the 1960 epidemic, which were 18.6 for *Ae. aegypti* and 0.8 for *Ae. albopictus* (Rudnick & Chan, 1965).

The present data suggest that in addition to *Ae. aegypti*, the closely related *Ae. albopictus* was also a vector of dengue haemorrhagic fever in Singapore. The relative importance of the two species in the transmission of the disease could not be assessed from the available information. The participation of the two species in the transmission of dengue haemorrhagic fever has also been observed in an epidemic on the island of Koh Samui on the western side of the Gulf of Thailand (Gould et al., 1968).

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RÉSUMÉ

AEDES AEGYPTI (L.) ET *AEDES ALBOPICTUS* (SKUSE) DANS LA VILLE DE SINGAPOUR: 5. OBSERVATIONS EN RAPPORT AVEC LE SYNDROME DENGUE/FIÈVRE HÉMORRAGIQUE

Il est difficile d'évaluer l'incidence réelle du syndrome dengue/fièvre hémorragique à Singapour étant donné que cette maladie n'est pas soumise à déclaration. Elle frappe surtout les habitants des quartiers urbains et les cas sont

particulièrement nombreux dans les secteurs à forte densité de population. Ces secteurs sont aussi ceux où les densités d'*Aedes aegypti* et d'*A. albopictus* sont les plus élevées, la première de ces espèces étant prédominante.

Des cas sont observés pendant toute l'année, mais on note des fluctuations saisonnières. Les données recueillies de 1966 à 1968 montrent que l'incidence de la maladie augmente à partir d'avril, atteint un clocher en novembre, puis diminue jusqu'en avril de l'année suivante où se marque de nouveau une recrudescence. Cette courbe épidémique correspond dans l'ensemble aux fluctuations des populations d'*A. aegypti* et surtout d'*A. albopictus*.

Au cours de 1966, 6 virus de la dengue ont été isolés: 1 virus de type 2 à partir d'un lot d'*A. aegypti*; 1 virus

de type 1 et 4 virus de type 2 à partir de cinq lots d'*A. albopictus*. Les moustiques avaient été capturés pendant la période d'incidence croissante du syndrome dans quatre secteurs différents de la ville. On estime que le taux d'infection par le virus de la dengue atteignait 0,51 par 1000 moustiques pour *A. aegypti* et 0,59 pour 1000 pour *A. albopictus*.

Les auteurs concluent qu'*A. aegypti* et *A. albopictus* jouent tous deux un rôle dans la transmission du syndrome dengue/fièvre hémorragique à Singapour.

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